# Contents

**Edward G. Lakatta, M.D., Chief**

# Laboratory of Cardiovascular Sciences

Gerontology Research Center
Room 3-D-09
Phone 410-558-8202
Fax 410-558-8150

The Laboratory of Cardiovascular Sciences (LCS) was established in 1985 as an outgrowth of the Cardiovascular Section of the Clinical Physiology Branch. LCS is presently organized into three sections: Cardiac Function, Membrane Biology, and Behavioral Hypertension. The Cardiac Function Section, which comprised the entire LCS at its incipience, is organized into six functional units, each headed by a tenured or senior scientist. The Membrane Biology Section was formerly in the Laboratory of Biological Chemistry and was assimilated into the LCS at the request of their scientists in 1991. The Behavioral Hypertension Section was formerly part of the Laboratory of Behavioral Science and joined LCS in 1997.

The overall goals of the Laboratory of Cardiovascular Sciences are (1) to identify age-associated changes that occur within the cardiovascular system and to determine the mechanisms for these changes; (2) to study myocardial structure and function and to determine how age interacts with chronic disease states to alter function; (3) to study basic mechanisms in excitation-contraction coupling and how these are modulated by surface receptor signaling pathways in cardiac muscle; (4) to determine the chemical nature and sequence of intermediate reactions controlling the movement of ions through ionic channels and pumps present in myocardium, and how these are affected by aging and disease; (5) to determine mechanisms that govern behavioral aspects of hypertension; (6) to determine mechanisms of normal and abnormal function of vascular smooth muscle and endothelial cells; and (7) to establish the potentials and limitations of new therapeutic approaches such as gene transfer techniques. In meeting these objectives, studies are performed in human volunteers, intact animals, isolated heart and vascular tissues, isolated cardiac and vascular cells, and subcellular organelles.

Each section/unit independently conceptualizes and implements its research portfolio. Opportunities for collaboration among units/sections, however, are fostered and encouraged. In addition to independent work, substantial interaction occurs among scientists both within and between

the sections/units. The stimuli for such interactions originate from individual scientists and from the Lab Chief, who commits substantial energy to encourage (but not to demand) these research collaborations. Consequently, many of the LCS projects become multi-faceted, spanning a range from humans to molecules. Using this approach, scientists recognize that future research advances require the integration of discoveries within and among individual research areas. The networking among individuals within LCS also extends to individuals in other institutes within the NIH, academic institutions, and industry. We believe that such networking among individual facets of the biomedical research community is required for integration of discoveries that is tantamount to practical application of these research discoveries. The broad overall LCS mission permits tenured scientists, senior fellows, and new fellows appointed to the Lab to chose their specific research projects. In other words, individuals are most productive when working on projects on which they develop their own "passion." The resultant LCS environment has become somewhat unique: it is not strictly akin to a university department in which each individual dictates his/her mission and applies for individual funding in order to implement the proposed program; however, neither is the LCS environment strictly "mission oriented" in the sense that each individual is mandated to work on a given project in a "top down" design. The LCS environment is best described as a balance between the above approaches; and in the broad sense, the collective research output of the Lab can be considered to be a "bottom up" approach. Specifically, most projects originate at the investigator level but are coordinated by the Lab/Section/Unit Chiefs to achieve a meaningful mosaic within the broad framework of the Lab mission.

## Laboratory of Cardiovascular Sciences Staff

### Office of the Chief

| | |
|---|---|
| Edward G. Lakatta | Chief |
| Sunny Blackiston | Lead Secretary |
| Vanessa Day | Secretary |
| Sharon Wright | Secretary |
| Christina Link | Clerk |

### Instrumentation Core Unit

| | |
|---|---|
| Harold Spurgeon | Physiologist |
| Paul Pullen | Computer Specialist |

### Cardiovascular Gene Therapy Unit

| | |
|---|---|
| Maurizio Capogrossi | Unit Head |
| Mark Talan | Medical Officer |
| Luba Poliakova | Visiting Fellow |
| Xiao Wang | Visiting Fellow |
| Rita Peila | Visiting Fellow |
| Mario Guglielmi | Visiting Fellow |
| Simona Di Peppe | Visiting Fellow |
| Luis Gowdak | Visiting Fellow |
| Adam Groothius | Biologist |

### Human Cardiovascular Studies Unit

| | |
|---|---|
| Jerome Fleg | Section Head |
| Frances O'Connor | Statistician |
| Jeanette Wright | Medical Instrument Tech |
| Tomasz Rywik | Visiting Fellow |
| Peter Vaitkevicius | Guest Researcher |
| Susan Zieman | Guest Researcher |
| Nikita Gill | Special Volunteer |
| Loretta Lakatta | Special Volunteer |
| Kyle May | Guest Researcher |
| Kristin Bryant | Special Volunteer |

### Receptor Signalling Unit

| | |
|---|---|
| Rui-Ping Xiao | Unit Head |
| Ying Ying Zhou | Visiting Fellow |
| Ding-Ji Wang | IRTA |
| Meike Kuschel | Visiting Fellow |
| Sheng Jun Zhang | Guest Researcher |
| Leslie Heckendorf | Student IRTA |

### Molecular Cardiology Unit

| | |
|---|---|
| Kenneth Boheler | Unit Head |
| Lydia O'Neill | Biologist |
| Randy Stinson | Biologist |
| Huangtian Yang | Visiting Fellow |
| Gang Wang | Visiting Fellow |
| Ellen Vieyra | Special Volunteer |
| Robert Wade | Guest Researcher |

### Excitation-Contraction Coupling Unit

| | |
|---|---|
| Edward Lakatta | Unit Head |
| Harold Spurgeon | Physiologist |
| Michael Stern | SBRS Senior Investigator |
| Andrzej Janczewski | Visiting Associate |
| Bruce Ziman | Biologist |
| Steven Sollott | Investigator |
| Martin Villa Petroff | Visiting Fellow |
| Dennis Rozanski | IRTA |
| Su Wang | IRTA |
| Heping Cheng | Senior Staff Fellow |
| Ye Chen | Staff Fellow |
| Long-Sheng Song | Visiting Fellow |
| Jonathan Lederer | Guest Researcher |
| Renuka Misra | Guest Researcher |
| Roy Ziegelstein | Guest Researcher |
| Jeffrey Dodd-o | Guest Researcher |
| Quinghau Hu | Guest Researcher |
| Gemin Jerry Zheng | Special Volunteer |
| K. Shivakumar | Guest Researcher |
| James Sham | Guest Researcher |

**Vascular Studies Unit**

| | |
|---|---|
| Michael Crow | Unit Head |
| Robert Monticone | Biologist |
| Yehezkiel Gluzband | Chemist |
| Karen Curto | NRC Fellow |
| Ondrej Juhasz | NRC Fellow |
| Martha Lundberg | NRC Fellow |
| Renee Peralta | Pre-IRTA |
| Nick Papadopoulos | Guest Researcher |
| Toshinobu Asai | Guest Researcher |

**Membrane Biology Section**

| | |
|---|---|
| Jeffrey Froehlich | Section Head |
| Linda Cheng | Research Chemist |
| James Kinsella | Research Physiologist |
| Phillip Heller | Research Chemist |
| G. Mark Jenkins | Guest Researcher |

**Behavioral Hypertension Section**

| | |
|---|---|
| David Anderson | Section Head |
| Olga Federova | Visiting Associate |
| Daniel Parsons | Psychologist |
| Itharat Prat | IRTA |
| Christina Eyster | IRTA |
| Apsara Dhokalia | Special Volunteer |

**Edward G. Lakatta, M.D.**
Chief of the Laboratory and
Head, Cardiac Function Section

Gerontology Research Center
Room 3-D-09
Phone 410-558-8202
Fax 410-558-8150
E mail lakattae@grc.nia.nih.gov

**Keywords:**
cardiovascular aging
G protein coupled
cardiac receptors
cardiac apoptosis
vascular cell chemotaxis

**Recent Publications:**

Sollott SJ, et al. *Am J Physiol 271* 1996; 40: H896-H905.

Schulman SP, et al. *Circ* 1996; 94: 359-367.

Shah AM, et al. *Circ Res* 1997; 80: 688-698.

Pepe S, et al. *Circ* 1997; 5: 2122-2129.

**Biography:** Dr. Lakatta received his M.D. from Georgetown University School of Medicine, Washington, D.C. in 1970. His postdoctoral training included an internship and residency in medicine at Strong Memorial Hospital, University of Rochester School of Medicine; cardiology fellowships at Georgetown and Johns Hopkins University Hospitals; and basic research training at NIH and at the Department of Physiology, University College, London, England. He was section chief of the Cardiovascular Laboratory in the Clinical Physiology Branch from 1976 until 1985, at which time he founded the Laboratory of Cardiovascular Sciences.

**Cardiac Function Section Program**

Dr. Lakatta directs the Cardiac Function Section (CFS) which has a broad based research program ranging from studies in humans to molecules. The program is comprised of the following units:

**Human Cardiovascular Studies Unit:** This unit's studies deal with the interactions of age, lifestyle, and disease on cardiovascular structure/function in humans. The study panel for the bulk of the studies is the Baltimore Longitudinal Study of Aging (BLSA). Initially, age-associated changes in cardiovascular structure and function are defined in healthy individuals and subsequent studies define mechanisms for these changes. Additional populations that provide a diversity of lifestyle and disease have been added to the study panel for specific projects. Acute or chronic interventions in these individuals or in the BLSA are utilized to determine the responsiveness of age-associated changes to pharmacological therapies or lifestyle changes, for example, exercise habits. Several areas of related research in animal tissue and cells implemented in other units of the Section complement these studies in humans.

**Molecular Cardiology Unit:** The main focus of this unit is to define the molecular bases of structure-function changes in the heart with aging. Regulation of cardiac cell growth and size is a major area of study within

this Unit, as one hallmark of advancing age is an increase in cardiac cell size. Many features of the *pattern* of age-associated changes in heart cells resemble the hypothyroid state. Thus, regulation of intracellular thyroid and retinoid (RxR) receptors has become a recent focus of this unit. As heart failure increases exponentially with age, studies of the transition from compensated cardiac hypertrophy to heart failure in animals of old age with hypertensive heart disease have been initiated. The focus of additional studies is early cardiac gene expression using an embryonic stem (ES) cell differentiation model system. In these studies, potential early cardiac gene transcription factors are identified and the proteins responsible for activating expression are sought using standard molecular biological techniques. These factors are then cloned and their role in regulating cardiac gene expression examined with respect to their potential contribution to the aging process.

**Excitation-Contraction Coupling Unit:** This unit's main research focus is on the control of cardiac cell regulation. Substantial evidence indicates that the triggering of sarcoplasmic reticulum calcium release in cardiac muscle depends upon the interaction of the L-type sarcoplasmic calcium channel (dihydropyridine receptor) and the sarcoplasmic reticulum (SR) calcium release (ryanodine receptor) via local calcium gradients. This unit has developed quantitative mathematical models that embody this "local control" hypothesis. To test the predictions of these models, we require the ability to alter the behavior of these channels, while preserving their natural geometrical relationship in the cardiac myocyte. To achieve this, models are developed in which the relevant proteins (DHPR, RyR, FKBP-12.6) are mutated by homologous recombination in mouse embryonic stem cells. Genetically engineered myocytes produced are studied by biophysical techniques (patch-clamp and confocal microscopy). Additional projects deal with identifying how cardiac cell regulatory mechanisms become altered with aging and disease (anoxia, ischemia, hypertension, heart failure). The initial mechanisms focus of this unit has broadened from the study of biophysical mechanisms in cardiac cells to endothelial and vascular smooth muscle cells (VSMC) as well. These studies, which combine fluorescence and confocal imaging, link strongly to projects within the Vascular Studies Unit.

**Receptor Signalling Unit:** The unit's focus is on elucidating distinct signal pathways for α and ß receptor subtypes and of opioid signal transduction pathways in the heart. The interaction of signals emanating from stimulation of these with other receptor-mediated signaling pathways

is also studied. Studies are designed to integrate information gleaned from electrophysiology, UV fluorescence, and confocal imaging, and probe novel intracellular regulatory mechanisms.

**Gene Therapy Unit:** Investigators in the unit engineer expression cassettes for insertion into non-replicative adenoviruses, which are then utilized in experiments to deliver genes to promote angiogenesis or to reduce restenosis following angioplasty. Growth factors, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF), and the metalloproteinase inhibitor, tissue inhibitor of metalloprotinease (TIMP), have been the initial thrust of this effort. The Gene Therapy Unit interacts with other LCS units/sections, serves as a resource for other GRC labs, and collaborates with industry and academic institutions in animal trials that employ gene targeted therapy.

**Vascular Studies Unit:** Research areas of this unit include matrix regulation of the differentiation status of vascular smooth muscle cells (VSMC), characterization of VSMC properties (migration, secretion, invasion) of dedifferentiated (modulated) *in vivo*, i.e., from neointimal lesions in restenosis injury, or from atherosclerotic plaque, and *in vitro*, i.e., in VSMC cells in tissue culture and various aspects of extracellular matrix remodelling. A major focus is directed at discovering novel aspects of growth factor receptor-coupled signaling pathways that regulate cell migration, and how these pathways change with age. Similar studies on signaling mechanisms of advanced glycation end-products (AGE) via their receptors (RAGE) on VSMC form an additional facet of the Unit's work. This Unit is highly interactive with other parts of a LCS-wide "vascular initiative" composed of Gene Therapy and Excitation-Contraction Coupling and Human Studies Units within the Cardiac Function Section of the Membrane Biology Section. The Vascular Unit also networks widely with academic institutions and industry.

**Collaborators:** Maurizio C. Capogrossi, M.D., Jerome L. Fleg, M.D., Rui-Ping Xiao, M.D., Ph.D., Kenneth R. Boheler, Ph.D., Michael Crow, Ph.D., IRP, LCS; Steven Houser, Ph.D., Temple University School of Medicine; Brian Kobilka, M.D., Stanford University; Robert Lefkowitz, M.D., and Walter Koch, Ph.D., Duke University Medical Center; Remesh Gopal, MBBS, Northwestern University; Ajay Shah, M.D., University of Wales College of Medicine; Konstantin Bogdanov, M.D., Russian Academy of Medical Sciences; Gary Gerstenblith, M.D., Edward Shapiro, M.D., Frank Yin, M.D., and Peter Vaitkevicius, M.D., Johns Hopkins Medical School; Ruth Altschuld, Ph.D., Ohio State University; W. Jonathan Lederer, Ph.D., University of Maryland School of Medicine.

**Maurizio C. Capogrossi, M.D.**
Chief, Gene Therapy Unit

Gerontology Research Center
Room 3-111
Phone 410-558-8645
Fax 410-558-8150
E mail capogrossi@grc.nia.nih.gov

**Keywords:**
p53
apoptosis
gene therapy
angiogenesis

**Publications:**

Gorospe MC, et al. *Oncogene* 1997; 14: 929-935.

Pierzchalski P, et al. *Exp Cell Res* 1997; 234: 57-65.

Pili R, et al. *Cardiovasc Res* , In press.

Safi J, Jr. et al. *J Mol Cell Cardiol* 1997; 29: 2311-2325.

**Biography:** Dr. Maurizio C. Capogrossi received his M.D. from the University aLa Sapienza in Rome in 1975. One year later he moved to the United States to obtain further training. He was an Intern and a Resident in the Internal Medicine program at Emory University in Atlanta, Georgia. In 1982 he became a Staff Fellow in the Clinical Physiology Branch at the Gerontology Research Center and subsequently he was a Cardiology Fellow in the Johns Hopkins University program. In 1987 he became a tenured NIA Investigator and a faculty member in the department of Internal Medicine (Cardiology Division) at Johns Hopkins University. Dr. Capogrossi's research work has focused on the physiology of myocardial and endothelial cells. Since 1993 his interest has shifted to gene therapy to induce therapeutic angiogenesis and inhibit neointima development after vascular injury.

**Gene Therapy To Induce Therapeutic Angiogenesis:** The broad objective of this program is to perform tissue culture and preclinical experiments in animal models of myocardial and hindlimb ischemia to evaluate the therapeutic potential of gene therapy with angiogenic growth factors. Vascular cell's response to angiogenic growth factors, acidosis and hypoxia may help identify which strategy is more likely to induce therapeutic angiogenesis. *In vivo* experiments are aimed at characterizing clinically relevant animal models and at evaluating whether, and eventually under which conditions, adenovirus-mediated gene transfer of angiogenic growth factors induces therapeutic angiogenesis.

**Synergistic Effect of IGF-1 and bFGF on Microvascular Endothelial Cell Proliferation:** The effect of IGF-1 on endothelial cell function is poorly characterized and the objective of this study was to determine the effect of IGF-1 alone and in conjunction with bFGF and VEGF on the proliferation of human umbilical vein endothelial cells (HUVEC) and human microvascular endothelial cells (HMVEC). IGF-1 alone did not induce proliferation of either HUVEC or HMVEC and there was no

synergy between IGF-1 and VEGF on either HUVEC or HMVEC proliferation. In contrast IGF-1 had a marked synergistic effect with bFGF on HMVEC.

**Effect of Acidosis on Bovine Aortic Endothelial Cells Proliferation and Growth Factors Release:** Tissue ischemia stimulates angiogenesis and is associated with intracellular and extracellular acidification. However, the effect of acidosis on endothelial cell function is still unclear. We evaluated whether hypercarbic acidosis modulates bovine aortic endothelial cell (BAEC) proliferation and growth factor release into the conditioned medium. We found that acidosis inhibits BAEC proliferation in 10% FCS. Further, acidosis enhances growth factor release from BAEC and this is associated with increased cell proliferation when BAEC are cultured under starving conditions.

**Hypoxia Modulates Integrin Expression in Endothelial Cells:** Ischemia modulates endothelial function and stimulates new blood vessel growth. Since $\alpha_v ß_3$ and $\alpha_v ß_5$ integrins are involved in the angiogenic process we evaluated the effect of hypoxia on endothelial cell expression of these integrins. In addition, $ß_1$ subunit expression was assessed. HUVEC in complete medium were cultured in hypoxic conditions ($pO_2$<15 torr) for 24, 48, or 72 hours. Hypoxia had a significant effect to decrease $\alpha_v ß_5$ expression at all time points; $\alpha_v ß_3$ was not significantly different from normoxic control at 24 and 48 hours while it exhibited a 20% decrease which did not achieve statistical significance at 72 hours. $ß_1$ integrin expression remained unchanged under hypoxic conditions. Thus, hypoxia selectively decreases $\alpha_v ß_5$ expression and this may play a role in the angiogenic response to ischemia.

**Age-Dependence of Muscle Bioenergetic Recovery After Acute Ischemia:** We hypothesized that recovery of skeletal muscle metabolism after acute ischemia may be age-dependent. Male rats 2 and 20 months old underwent right femoral artery removal. Gastrocnemius muscle bioenergetics was evaluated by $^{31}P$ NMR on the operated and unoperated hindlimb 1 and 7 days after surgery. Peak heights of the phosphocreatinine (Pcr) and inorganic phosphate (Pi) resonances were obtained at 2 min intervals, at rest, during a 6 min period of electrical stimulation of the hindlimb and during at 12 min period of rest. PCr/(PCr+Pi) peak height ratios were evaluated prior to stimulation ($R_0$), when the ratio achieved a minimal value ($R_{in}$), and at the end of the recovery period ($R_{end}$). PCr/(PCr+Pi) ratios of the unoperated limbs were indistinguishable between groups and exhibited less decline with stimulation and faster recovery after stimulation than the operated limbs. In contrast, results for the operated limb show that 1 day after surgery both $R_0$-$R_{min}$ and $R_{end}$-$R_{min}$ are lower in

old than in young rats ($p<0.005$). Thus, following acute ischemia, muscle bioenergetic recovery is impaired in old vs. young rats.

**Adenovirus-Mediated Gene Transfer and Biosafety - Lack of Tumorigenicity Upon Transient versus Permanent Expression of Secreted and Non-Secreted Forms of Acidic Fibroblast Growth Factor:** Gene transfer of endothelial growth factors is being implemented as a possible therapeutic approach for the treatment of ischemic disorders. However, the role of growth factors on tumor growth has raised a biosafety issue. We tested the replication-deficient recombinant adenovirus (Ad) vectors coding for the signal sequence (Ad.CMV.sp+aFGF) and non-signal sequence (Ad.CMV.aFGF) forms of human acidic fibroblast growth factors both *in vitro* and *in vivo*. The results showed that phenotypic changes induced by adenovirus-mediated gene transfer of aFGF are transient, suggesting that transient expression of growth factors might induce only a transitory growth advantage, but not a stable transformation of normal cells.

**Adenovirus-Mediated Acidic Fibroblast Growth Factor Gene Transfer Increases Arteriole Density and Reduces the Risk Region for Myocardial Infarction in Rabbits - Evidence of Induction of Angiogenesis in the Non-Ischemic Heart:** The majority of patients with severe coronary artery disease have normal baseline myocardial blood flow. Therefore, interventions aimed at inducing therapeutic angiogenesis in these patients should cause new blood vessel growth in the heart in the absence of chronic ischemia. It was examined whether adenovirus-mediated gene transfer of acidic fibroblast growth factor ($aFGF_{1-154}$) in a discrete area of non-ischemic myocardium, next to a major epicardial artery, may induce neovascularization and whether by this approach it is possible to reduce the risk region for myocardial infarction upon coronary ligation near the injection site. The results showed that gene therapy with AdCMV.sp+$aFGF_{1-154}$ can induce angiogenesis in a discrete but critical area of myocardium in the absence of chronic ischemia. The newly formed collateral blood vessels provide anatomical basis for the reduction in the risk region for myocardial infarction upon subsequent coronary artery occlusion.

**Gene Therapy to Inhibit Intimal Hyperplasia After Endovascular Injury:** Vascular smooth muscle cells (VSMCs) play a major role in the arterial wall response to injury. VSMCs are normally present in the arterial tunica media where they regulate vascular tone and blood flow. In the vessel wall, VSMCs are surrounded and separated from other cells by extracellular matrix (ECM). Arterial injury leads to proliferation of medial VSMCs and migration of these cells from the media to the intima. These steps are dependent on the local degradation and remodeling of the ECM.

Our studies examined the role of adenovirus (Ad)-mediated wild-type p53 (AdCMV.p53) and tissue inhibitor of metalloproteinase 2 (AdCMV.hTIMP-2) overexpression in vascular smooth cells (VSMCs). In preliminary experiments AdCMV.p53 failed to induce VSMC apoptosis *in vitro* and inhibition of intimal hyperplasia in the rat model of neointima development after carotid injury. In contrast, AdCMV.p53 induced melanoma cell apoptosis. Therefore, the role of p21, a p53-effector gene was examined in the different responses of VSMC and melanoma cells to AdCMV.p53. The results show that the failure of AdCMV.p53 to induce VSMC apoptosis is associated with enhanced p21 expression in these cells. In contrast, in melanoma cells AdCMV.p53 results in apoptosis and does not increase p21. In additional experiments it was shown that Ad-mediated p21 overexpression before exposure to AdCMV.p53 protects melanoma cells from the apoptotic effect of this viral vector. The results support the view that p21 plays a fundamental role in the decision fork between programmed cell death and survival and account for the failure of AdCMV.p53 to inhibit neointima development. In a different study, the effect of AdCMV.hTIMP-2 on VSMC function *in vitro* and on neointimal development *in vivo* was assessed. Previous studies in the rat model of carotid injury indicated that vascular injury increases activation of matrix metallo-proteinase 2 (MMP2) during the time VSMCs migrate to the intima. TIMP-2 is a physiologic MMP2 inhibitor and AdCMV.hTIMP-2 inhibits MMP2 activity and SMC invasion in cultured VSMC. In this study, 6 month old rats underwent balloon injury of the common carotid artery. The vessel wall was infected either with AdCMV.hTIMP-2 or with the control vector AdCMV.null at the time of balloon injury. AdCMV.hTIMP-2 transgene expression in VSMCs *in vivo,* was shown by immunohistochemistry 5 days after injury and infection. At 4 days post-injury and infection, intimal cell number was decreased by 36% in AdCMV.hTIMP-2 *vs* AdCMV.null infected carotid arteries. At 8 days after injury and infection, AdCMV.hTIMP-2 induced a 50% reduction of neointimal area *vs* AdCMV.null. In contrast to the changes seen in the neointima, no difference was seen in medial wall area. Thus, Ad-mediated TIMP-2 overexpression in the rat model of carotid artery injury inhibits VSMC migration and decreases the severity of neointimal formation.

**Collaborators:** I. Kovesdi, GenVec, Inc., Rockville, Maryland; P. Anversa, New York Medical College, New York; L. Becker, Johns Hopkins University, Baltimore, Maryland; W. Stetler-Stevenson, NIH, Bethesda, Maryland; A. Felici, Instituto Dermopatico dell' Immacolata, Rome, Italy; Richard Spencer, Kenneth Fishbein, Kimberlee Potter, and Pavel Shkarin, Laboratory of Cellular and Molecular Biology, NIA.

**Jerome L. Fleg, M.D.**
Head, Human Cardiovascular Studies Unit

Gerontology Research Center
Room 3-C-19
Phone 410-558-8206
Fax 410-558-8150
E mail jfleg@vax.grc.nia.nih.gov

**Keywords:**
aging
exercise physiology
silent ischemia
heart failure

**Recent Publications:**

Byrne E, et al. *J Appl Physiol* 1996; 81: 743-750.

Schulman S, et al. *Circulation* 1996; 94: 359-367.

Swinne CJ, et al. *Am J Cardiol* 1996; 78(9): 1070-1073.

Pearson JD, et al. *J Gerontol: Med Sci* 1997; 52A: M177-M183.

**Biography:** Dr. Jerome Fleg received his M.D. from the University of Cincinnati in 1970. After completing training in Internal Medicine and Cardiovascular Disease at Washington University in 1977, he assumed his current position in NIA's Laboratory of Cardiovascular Science. His research interests include normative aging changes in cardiovascular structure and function, silent myocardial ischemia, and congestive heart failure.

**Effects of Age, Gender, Lifestyle and Disease on Cardiovascular Structure and Function**: Advancing age in humans is accompanied by significant changes in the cardiovascular system and, all too often, by the development of cardiovascular disease. A major challenge undertaken by our laboratory is to define normative aging changes in cardiac and vascular structure and function and their modulation by lifestyle variables and disease. To accomplish this ambitious task, we utilize a wide variety of noninvasive testing methodologies at rest and during exercise.

Early M-mode echocardiographic studies in our laboratory, pioneered by Drs. Gary Gerstenblith and Edward Lakatta, demonstrated that normative aging was accompanied by a thickening of the left ventricular (LV) muscular wall and a reduction of early mitral value closure slope analogous to the findings in mild hypertension. These findings have led us to conceptualize that aging is a muted form of hypertension. In industrialized societies, a 20-30 mm Hg rise in systolic blood pressure (SBP) typically occurs across the adult lifespan in subjects who remain normotensive by clinical criteria. The etiology of this SBP rise involves a gradual replacement of elastic fibers in the vascular media by less distensible collagen and calcium. Recent studies in our laboratory are quantifying these age-associated changes in arterial stiffness using pulse wave velocity and applanation tonometry of the large arteries. These studies have demonstrated a 200-500% increase in stiffness across the adult life span. Two-dimensional echocardiographic determination of LV mass in these same subjects has

revealed that arterial stiffness, especially the late systolic augmentation of arterial pressure quantified by applanation tonometry, is an independent determinant of LV mass, beyond the effect of SBP. These studies, therefore, support the hypothesis that age-associated increases in arterial stiffness are responsible in part for the mild LV hypertrophy and substantial reduction in early diastolic LV filling rate seen with aging. To test this hypothesis, we have designed short-term drug interventions and longer-term exercise training interventions to determine whether arterial stiffness can be reduced, both in normal older subjects and individuals with congestive heart failure. Although the exercise training studies are still in progress, a recently completed study has shown that acute infusion of the vasodilator sodium nitroprusside to normal older subjects dramatically reduced their resting arterial stiffness and improved their LV performance during exhaustive cycle exercise to levels typical of unmedicated young individuals.

Another major goal of our laboratory is to determine the mechanisms for the well known decline in maximal aerobic capacity ($VO_{2max}$) seen with aging. In an early study, we found that normalization of treadmill $VO_{2max}$ for total body muscle mass nearly eliminated the age-associated reduction in $VO_{2max}$, inferring that the loss of muscle tissue with age contributes importantly to the decline in $VO_{2max}$. We have employed gated cardiac blood pool scans with the isotope technetium-99m to quantify LV performance at rest and during maximal upright cycle exercise and its modulation by age, gender, lifestyle variables and cardiovascular disease. Our initial investigation using this techniques demonstrated that stroke volume at peak exercise was preserved across age by a greater reliance on LV dilatation to compensate for reduced systolic emptying. More recently we have found that this age-associated LV dilatation during exercise is more prominent in men than women despite similar impairment in emptying. Endurance trained older subjects utilize both larger end-diastolic LV volumes and enhanced LV emptying to augment stroke volume during exercise to a greater degree than untrained individuals.
Simultaneous monitoring of oxygen consumption throughout these exercise cardiac blood pool scans has allowed us to examine the relative importance of cardiac versus peripheral factors in the age-associated decline in aerobic capacity and its modulation by endurance training. A recent investigation using this methodology suggests that declines in cardiac output and arteriovenous oxygen difference contribute nearly equally to this decline in aerobic capacity with aging. Similarly, the marked augmentation of peak $VO_2$ in endurance trained older subjects relative to their sedentary peers is accomplished to a similar extent by enhanced cardiac output and peripheral oxygen extraction.

We have also utilized pharmacological probes to further define mechanisms for the decline in maximal exercise cardiac performance with age and their potential for modulation. For example, beta adrenergic blockade during exhaustive cycle ergometry in younger subjects markedly reduced their maximal heart rates and systolic emptying and augmented their exercise-induced LV dilatation, producing a profile similar to that of older unmedicated subjects. These data support our hypothesis that an important mechanism for the age-associated reduction in maximal cardiac performance is reduced beta adrenergic responsiveness.

**Collaborators:** Edward Shapiro, M.D., Gary Gerstenblith, M.D., Lewis Becker, M.D., Steven Schulman, M.D., Johns Hopkins University; Leslie Katzel, M.D., Andrew Goldberg, M.D., University of Maryland at Baltimore; James Hagberg, Ph.D., Stephen Porges, Ph.D., University of Maryland, College Park; Yoji Nagai, M.D., E. Jeffrey Metter, M.D., NIA.


**Michael T. Crow, Ph.D.**
Head, Vascular Studies Unit

Gerontology Research Center
Room 3-C-13
Phone 410-558-8207
Fax 410-558-8150
E mail crowm@grc.nia.nih.gov



**Keywords:**
heart
vascular smooth muscle
cell migration
apoptosis


**Biography:** Dr. Michael Crow received his Ph.D. in Physiology and Biophysics from Harvard University in 1981 and did postdoctoral studies in cellular and molecular biology of skeletal muscle development at Stanford University. In 1984, he joined the Faculty of the Department of Pharmacology at the University of Texas, Houston and moved to his current position in the NIA in 1991, shifting research interests from skeletal muscle to smooth muscle and cardiomyocyte cellular and molecular biology.

**Vascular Smooth Muscle Cell Biology**: We study the behavior of isolated vascular smooth muscle cells (VSMCs) and cardiomyocytes. In the past year, our work has been concentrated on three distinct areas.

**Intracellular Signaling Pathways Regulating VSMC Migration**: The migration of vascular smooth muscle cells (VSMCs) is a key event in the pathogenesis of many vascular diseases. Migration of resident VSMCs

**Recent Publications:**

Bilato CB, et al. *J Clin Invest* 1997; 96: 1905-1915.

Bilato CB, et al. *J Clin Invest* 1997; 100: 1-12.

Long X, et al. *J Clin Invest* 1997; 99: 2635-2643.

Pauly RR, et al. Methods in Cell Biology (Eds.) HL Sweeney and C Emerson, *Academic Press,* San Diego, CA 1998; 52: 133-154.

requires that the cells undergo a phenotypic switch from a contractile to synthetic/proliferative state. We previously showed that a key factor in this switch was the ability of VSMCs to activate the multifunctional protein kinase, calcium/calmodulin-dependent protein kinase II (CamKII). Our current work is focused on identifying the intracellular targets for CamKII, its upstream regulation, and its unique role in ß3 integrin-mediated signaling of ß1 integrin function. We have shown that nonmuscle myosin light chain kinase is inhibited by CamKII and that this inhibition is important to the mechanism by which CamKII regulates PDGF-directed migration. In addition, we have shown that platelet derived growth factor (PDGF)-stimulated CamKII occurs through a signaling pathway different than that employed by other receptor agonists and requires the small GTPase protein, $p21^{rac}$, and the generation of reactive oxygen species. This unique pathway for activating CamKII provides additional nodes at which migration can be regulated by the availability of co-stimulatory growth factors, such as basic fibroblast growth factor (bFGF). Recently, we demonstrated that occupancy of ß3 integrin complexes is also required for CamKII activation and VSMC migration and that signaling from ß3 integrins to CamKII occurs through a bFGF-dependent signaling pathway. Occupancy of ß3-containing integrins in VSMCs not only regulates migration by facilitating CamKII activation but also by suppressing non-integrin signaling pathways for migration. In fact, the migration of VSMCs that lack functional ß3 integrins, such as those from aged animals, is not regulated by CamKII and may possibly underlie the exaggerated response of aged vessels to endothelial denudation. Our results represent the first demonstration of how outside-in signaling by ß3-containing integrins modulates a specific growth-factor stimulated signaling pathway. They identify a unique intracellular signalling network in VSMCs that is triggered by chemoattractant recognition and modulated by growth status, secretion of growth factors and ECM components, and ECM-VSMC interactions.

**Advanced Glycation Endproducts, Their Receptors, and Vascular Disease**: Advanced glycation endproducts of proteins (AGE) accumulate in the plasma and in tissues with age and at an accelerated rate in diabetes. In isolated vascular cells, AGEs induce a proxidant stress, leading to activation of pro-inflammatory events such as increased activity of MAPK and NF-kB, increased monocyte chemoattractant protein-1 (MCP-1) production, and increased PDGF B chain activity, all of which have been implicated in vascular lesion development. We have demonstrated that many of the effects of AGEs on gene expression are mediated through a unique immunoglobulin-type receptor called RAGE. We have constructed epitope-tagged wild type and mutant RAGE molecules and have shown that transfection of wild type receptor leads to increased MAPK activity

and MCP-1 RNA and protein levels in response to AGEs. Mutant receptors in which the cytosolic tail has been removed, however, do not result in increased MCP-1 production, but in fact block the ability of co-transfected wild type receptors to signal. These observations demonstrate that RAGE acts not merely as an AGE-binding protein but a bona fide transmembrane receptor, engaging intracellular signaling molecules to affect changes in gene expression and protein production and secretion. Current studies are concentrated on exploiting the truncated receptor as a dominant negative to block the effects of RAGE-mediated signaling during vascular lesion development in transgenic mice. In addition, interaction cloning techniques are being used to identify intracellular proteins associated with the receptor.

**Cardiomyocyte Apoptosis**: Cardiac cell loss marks the transition from hypertrophy to heart failure and is the likely result of chronic myocardial ischemia and cell hypoxia. Cell loss is due predominantly to the death of cardiac myocytes and is mediated in part by apoptosis. Because adult cardiac myocytes are terminally differentiated cells, the effects of such loss can never be fully compensated. The identification of the intracellular signaling events and extracellular factors that regulate this process and the development of strategies to prevent such loss is, therefore, likely to have important beneficial consequences. We have adopted an experimental system to induce cardiomyocyte cell death by apoptosis that involves exposing neonatal cardiomyocytes to prolonged hypoxia. We have shown that there is increased expression and transactivating ability by the tumor suppressor gene, p53, that accompanies the onset of apoptotic cell death in these cells. Forced expression of p53 with a recombinant adenoviral vector was sufficient to induce cardiomyocyte, but not cardiac fibroblast, apoptosis. Forced expression of p21/WAF1, a downstream target for p53 transactivation, also resulted in apoptosis, as did the incubation of normoxic myocytes with bafilomycin, an inhibitor of membrane-associated proton pumps which, in other cell types, leads to intracellular acidification. Apoptosis induced by p53, p21, and bafilomycin was effectively prevented or delayed by exposure to the hypertrophy-inducing factor, phenylephrine (PE), occurring through a PI3-K-dependent pathway. Our current studies are directed at understanding how p53/p21 engage the “death machinery” in cardiomyocytes and in developing viral vectors to counteract the effects of p53.

**Collaborators:** Maurizio Capogrossi, NIA; Piero Anversa, New York Medical College, Valhalla, NY; David Stern, Anne-Marie Schmidt, Columbia University, NY; Scott Blystone, Fred Lindberg, Washington Univ., St. Louis, MO; Jonathan Fox, Univ. of Pennsylvania, Philadelphia, PA.

**Kenneth R. Boheler, Ph.D.**
Head, Molecular Cardiology Unit

Gerontology Research Center
Room 3-E-02
Phone 410-558-8095
Fax 410-558-8150
E mail bohelerk@grc.nia.nih.gov

**Keywords:**
heart
development
calcium handling
proteins
molecular biology

**Recent Publications:**

Kaddoura S, et al. *Circ* 1996; 93: 2068-2079.

Wankerl M, et al. *J Mol Cell Cardiol* 1996; 28: 2139-2150.

Wong K, et al. *Circ* 1997; 96: 2239-2246.

Martin XJ, et al. *Mol Cell Biochem* 1996; 157: 181-189.

**Biography:** Dr. Boheler received his B.Sc. from Duke University and his Ph.D. from the University of California, San Diego. After completing a post-doctoral fellowship at Unit 127 of the National Institutes of Health and Medical Reseach (INSERM) in Paris, France, he was appointed Assistant Professor (Lecturer) at Imperial College School of Medicine in the Department of Cardiothoracic Surgery, London, United Kingdom. In October of 1996, he joined the NIH to head the Molecular Cardiology Unit of the Cardiac Function Section of the Laboratory of Cardiovascular Science.

**Research:** The focus of our research over the past several years has involved examination of the expression and regulation of a number of proteins involved in regulating calcium movements in cardiac myocytes, including the sarcoplasmic reticulum calcium ATPase (SERCA), phospholamban (PLB), the Na/Ca exchanger (NCX) and the sarcoplasmic reticulum calcium release channel (Ryanodine Receptor). The work has involved examination of the spatial and temperol expression of these mRNAs and proteins in the developing myocardium. Using simpler *in vitro* models, the regulation of presence of the mRNAs encoding some of these gene products has been studied through examination of signal transduction pathways. Isolation and characterization of the promoter regions for human SERCA2 gene and rat NCX are underway. And, in the case of cardiac hypertrophy, we have examined the effects of angiotensin converting enzyme inhibitors on their ability to improve diastolic dysfunction in the rat myocardium. Our recent work is focused on use of an *in vitro* differentiation model of embryonic stem cells and embryonic carcinoma cells in an attempt to further understand the consequences of development and of altered gene expression on function of these proteins.

**Spatial and Temporal Analyses:** These studies have been performed in close collaboration with the laboratory of Dr. Antoon Moorman, Amsterdam. With the development of molecular cell markers specific for contraction and relaxation, functional aspects of myocardial differentia-

tion have been addressed through the use of *in situ* hybridization. We have reported how expression of SERCA2 and PLB in the rat may partly explain why the embyronic atrium and ventricle function essentially as they do in the adult. SERCA2 is expressed in a craniocaudal gradient; whereas that of PLB is expressed in a gradient essentially opposite to that of SERCA2. Accumulation of the NCX and RyR transcripts also occurs very early, similar to that for SERCA2, but do not show gradients of expression. With development SERCA2 and PLB expression increase during late fetal and perinatal development; whereas that for NCX decreases at or around birth in a compartment dependent manner. Its expression is however increased with aging.

**Signal Transduction Pathways Mediating SERCA2 and PLB Expression:** Using a model of neonatal rat cardiomyocytes, we have been able to determine that adrenergic agonists can play a critical role in regulation SERCA2 and PLB mRNA accumulations. The pathways have some overlap, in that activation by $\alpha$ adrenergic agonists and protein kinase C isoforms reduces both their expressions in a time and dose dependent mechanism probably through activation of the MAP kinase system. Beta adrenergic activation only results in decreased SERCA2 mRNA expression through a pathway that requires extracellular calcium and entry via the voltage dependent sarcolemmal calcium channel. The regulation also appears to be primarily transcriptional based on transfection data of the human SERCA2 genomic constructs linked to reporter sequences.

**Expressional Analysis of Cardiac NCX in Development and Senescence:** We have examined the mRNA expression of the Na/Ca exchanger (NCX) in rat heart during perinatal development and with aging. NCX is highly expressed in late fetal and neonatal rat hearts, decreasing to adult levels by 20 days after birth. The lowest level of accumulation is seen in 6 and 18 month old animals. In the 24 month old senescent rat, NCX expression is increased by almost 50% above that seen at 6 and 18 months ($p<0.05$) but is not different from that at 15 neonatal days. Results from nuclear run-on assays indicate that NCX expression during the perinatal period is regulated at least partially through transcriptional mechanisms. Relatively high transcriptional activity is seen at birth but by 20 post-natal days, no transcriptional activity from NCX can be detected. During development, there are no major changes seen in the use of the five identified transcription start sites, nor is there any major difference in the splicing patterns seen in the 5' untranslated regions. We have identified the presence of 5 different splicing variants in the cytosolic loop of the coding region, three of which have not been previously described in heart. We have also recently cloned a 2.8 kb fragment containing the putative cardiac NCX1 promoter and a consensus thyroid hormone responsive element which we are now examining.

**Embryonic Stem Cells and Myocardial Development:** This new research area involves a model of *in vitro* differentiation of cardiomyocytes originating from embryonic stem cells (R1) and embryonic carcinoma cells (P19). The research is aimed at understanding the developmental processes involved in cardiac myocyte differentiation and development. We are differentiating pluriopotent ES and EC cells in the presence of various growth factors to monitor the development of atrial versus ventricular like cells. To identify atrial versus ventricular like cells, expression vector constructs are being made that link atrial and ventricular markers to the green florescence protein (GFP). These constructs will be introduced into the cells and positive transformants identified through neomycin resistance selection. In concert with this project is the analysis of various promoter regions of α and beta myosin heavy chain, human SERCA2 and rat NCX as we attempt to determine which transcription factors are responsible for their induction at specific times of development. From this work, we hope to use various molecular techniques to identify and analyze various transcription factors and growth factors that promote cardiac cell division and differentiation and importantly, the sequence of their activation and inhibition.

**Collaborators:** Professor Magdi H. Yacoub, Imperial College School of Medicine, United Kingdom; Professor Antoon F.M. Moorman, University of Amsterdam, The Netherlands; Professor Alan Williams, Imperial College School of Medicine, United Kingdom; Dr. Kenneth MacLeod, Imperial College School of Medicine, United Kingdom; Dr. Ketty Schwartz, INSERM 153, France; Dr. Anne-Marie Seymour, University of Hull, United Kingdom; Professor Antonio Zorzano, University of Barcelona, Spain; Dr. Thomas Eschenhagen, University of Hamburg, Germany; Dr. Anna Wobus, Institut fur Pflanzengenetik und Kulturpflanzenforschung, Germany.

**Michael D. Stern, M.D.**
SBRS Senior Investigator, Excitation-Contraction Coupling Unit

Gerontology Research Center
Room 3-D-06
Phone 410-558-8097
Fax 410-558-8150
E mail mikes@grc.nia.nih.gov

**Keywords:**
calcium signals
excitation-contraction coupling
ryanodine receptors
mathematical modeling

**Recent Publications:**

Stern, MD et al. *Biophys J* 1996; 70: 2100-2109.

Rios, E et al. *Annu Rev Biophys Biomol Struct* 1997; 26: 47-82.

Stern, MD et al. *J General Physiology* 1997; 110: 415-440.

**Biography:** Dr. Stern studied theoretical physics at Princeton and received an M.D degree from University of Pennsylvania. Following internship, he was a Staff Fellow in the Laboratory of Technical Development of the National Heart Lung and Blood Institute (NHLBI), where he invented a method to measure tissue microvascular blood flow using laser light scattering. Following an Internal Medicine residency at University of Michigan and Cardiology fellowship at Johns Hopkins, Dr. Stern joined the faculty in Cardiology at Johns Hopkins in 1981. His research on laser light scattering fluctuations in cardiac muscle, in collaboration with the Laboratory of Cardiovascular Science at GRC, led to the discovery that apparently resting heart muscle produces continuous, random asynchronous subcellular waves of contraction, which proved to be due to propagated calcium release from the sarcoplasmic reticulum. This led directly to his present interest in the basic mechanism of cardiac excitation-contraction coupling. His studies on the physiology of excitation-contraction coupling in single cardiac myocytes during extreme hypoxia led to the finding that reoxygenation injury is due to calcium shifts brought about by ionic conditions created during a vulnerable period of complete energy depletion. In parallel with this work, Dr. Stern carried out mathematic modeling of the basic mechanisms of sarcoplasmic reticulum calcium release. Based on this work he proposed, in 1992, the *local control* hypothesis of excitation contraction coupling, which has become the leading theory of this process. In 1996, Dr. Stern joined LCS full time as a member of the Senior Biomedical Research Service.

**Calcium Microdomain Signaling in Intracellular Communication**: The heartbeat is initiated by the release of calcium from stores in the sarcoplasmic reticulum (SR). It is now well established that the trigger for this release is the entry of a much smaller amount of calcium through voltage-controlled L-type calcium channels in the cell membrane. This is the mechanism of *calcium-induced calcium release* (CICR), which is known to be mediated by ryanodine receptors, which

are calcium sensitive calcium channels located in the membrane of the SR. Similar ryanodine receptors are located on intracellular calcium stores in a wide variety of cell types, where their function is not yet understood.

The release of SR calcium is a tightly controlled and smoothly graded function of the trigger calcium; this is paradoxical since CICR is an intrinsically self-reinforcing process which might be expected to lead to an all-or-none response. A possible resolution of the paradox is based on the fact that the L-type trigger channels and the SR release channels are known to be localized to opposite sides of the 15 nm dyad junctions between the cell membrane and the SR membrane. This means that the trigger for CICR is not whole cell calcium, but rather the local calcium microdomain generated in the neighborhood of the triggering channel. We have shown mathematically that the interaction between the stochastic gating of individual channels and the fluctuating calcium microdomains which they generate can give rise to smoothly graded and controlled calcium release in the whole cell aggregate, even though individual release events may be nearly all-or-none. This is the *local control* hypothesis, which implies that whole cell calcium release depends critically on the details of the gating and ion permeation of the trigger and release channels, and on the local geometrical relationship between them. Over the past several years, considerable evidence has accumulated showing that this is the case. We have recently constructed a similar local-control model of the role of CICR in skeletal muscle excitation-contraction coupling. This model successfully explains many paradoxical observations, and leads to the insight that collective behavior of mesoscopic arrays of calcium-coupled release channels, which we term *couplons*, may be the basic functional unit of EC coupling.

In order to test the local control hypothesis more definitively, we hope to develop a model in which the full machinery of excitation-contraction coupling (junctions, ryanodine receptors, L-type calcium channel, auxiliary junctional proteins) is expressed and in which the components and the signals that control their localization can be manipulated genetically. This is the major project of our laboratory at the present time  Since cardiac myocytes are terminally differentiated and non-dividing, they cannot be used directly. In general, cultured cell lines do not form SL/SR junctions even when expressing the channel proteins. Our present approach to the problem is to make use of the well developed technique of gene targeting in mouse embryonic stem (ES) cells, together with *in vitro*  differentiation of ES cells into embryoid bodies which contain beating cardiac myocytes. We have successfully established culture techniques which promote cardiac differentiation in a high percentage of embryoid bodies, and have demonstrated calcium sparks and waves, which are produced by RyR-mediated intracellular calcium release, in cells as early as 7 days of differ-

entiation. These studies will be continued to characterize the biochemistry, ultrastructure and EC coupling physiology of these cells. These baseline studies will define that model and lead to increased understanding of the development of cardiac-type EC coupling. We will then use homologous recombination methods to obtain cardiac myocytes in which the key protein domains responsible for calcium sensing and release, and others (such as the enormous 2 megadalton “foot process” of the ryanodine receptor) whose function is unknown, have been altered. More importantly, we hope to discover the signals which give rise to the organized geometrical structure of the dyad junction, and to alter it in order to test the sensitivity of coupling to geometry which is predicted by the local control theory. A combined approach utilizing ES cell techniques, confocal calcium measurement, patch clamp and mathematical modeling will be used.

Since ryanodine receptors are ubiquitous, it is likely that the insights gained from this program will be important for understanding the way in which spatial and temporal localization of intracellular calcium signals leads to their diversity of function in many cell types.

**Collaborators:** Heping Cheng, Kenneth Boheler, LCS; Eduardo Rios, Department of Physiology and Molecular Biophysics, Rush University; Phillip Palade, University of Texas, Galveston; Michal Horowitz, Hebrew University, Jerusalem.


**Rui-Ping Xiao, M.D., Ph.D.**
Head, Receptor Signalling Unit

Gerontology Research Center
Room 3-D-13
Phone 410-558-8662
Fax 410-558-8150
E mail xiaor@grc.nia.nih.gov



**Keywords:**
2-adrenergic receptor
cAMP dependent protein kinase
pertussis toxin-sensitive G proteins
cardiac contractility


**Biography:** Dr. Rui-Ping Xiao has been working in the Laboratory of Cardiovascular Sciences since February, 1990. She was trained as a physiologist and pharmacologist at Tong-Ji Medical University, China, and at the University of Maryland, where she received her M.D. and Ph.D., respectively. Her scientific focus has been related to receptor-mediated transmembrane signal transduction in the cardiovascular system. The mechanistic and multidisciplinary nature of her research has made the past few years particularly fruitful. The breadth of Dr. Xiao’s work covers four different areas:

**Recent Publications:**

Xiao R-P, *Am J Physiol* 1997; 272: H797-H805.

Pepe S, *Circulation* 1997; 95: 2122-2129.

Xiao R-P, *J Physiol* 1997; 500: 331-342.

Zhou Y-Y, *Am J Physiol* 1997; 273: H1611-H1618.

Korzick DH, *Am J Physiol* 1997; 272: H590-H596.

1) Signal transduction mechanisms which underlie the distinct actions of ß-adrenergic receptor (ßAR) subtype stimulation in cardiac myocytes; 2) age- and heart failure-related alterations in cardiac responses to ßAR subtype stimulation; 3) interaction of the ß-adrenergic signalling pathway with other cardiac sarcolemmal receptor mediated signaling pathways (e.g., opioid, adenosine, and acetylcholine receptors); and 4) the physiological role of protein kinase-phosphatase in cardiac functional regulation (e.g., regulation of cardiac calcium influx via L-type calcium channels by Ca/calmodulin-dependent kinase or cAMP-dependent kinase).

Our recent studies have systematically documented the distinctly different cardiac response to $ß_2$- versus to $ß_1$-adrenergic stimulation. By taking advantage of genetic manipulations, including transgenic mice overexpressing cardiac $ß_2$ARs and $ß_1$AR or $ß_2$AR knockout models, we have revealed that cardiac $ß_2$AR couples to two functionally opposing G protein families, i.e., a stimulatory G protein and inhibitory G proteins, $G_{i2}$ and $G_{i3}$. The dual coupling of $ß_2$AR to $G_s$ and $G_i$ not only reveals a new level of complexity of cardiac ßAR signal transduction, but also provides new insights for understanding the physiological and pathophysiological significance of the differential regulation of ßAR subtypes. Because the diminished ßAR contractile response in failing or aging hearts is accompanied by a selective down-regulation of $ß_1$AR, without loss of $ß_2$AR, considerable effort has also been put on the potential role of $ß_2$AR activation for improving cardiac performance under these conditions. In canine and human failing cardiomyocytes, the efficacy of $ß_2$AR stimulation is markedly increased as compared to that of normal cells. The up-regulation of $ß_2$AR-directed signaling relative to $ß_1$AR may be beneficial, because it provides inotropic support without promoting sarcoplasmic reticulum (SR) $Ca^{2+}$ overload and spontaneous SR $Ca^{2+}$ release. The cellular logic for the multiplicity of ßAR may partly reside in the different arrhythmogenic properties of ßAR subtypes. In light of these recent findings, an increase in the ratio of $ß_2$AR/ $ß_1$AR and $G_i$ amount might reflect important adaptive changes associated with heart failure and cardiac aging. In addition, the novel signaling mechanism of $ß_2$AR stimulation, i.e. coupling of $ß_2$AR to $G_i$ proteins, represents a potential target for therapeutic interventions to attenuate the inhibitory pathway thereby extending and augmenting the action of various therapeutic agents.

**Collaborators:** Dr. Robert J. Lefkowitz, Howard Hughes Medical Institute; Dr. Walter J. Koch, Duke University Medical Center; Dr. Ruth Altschuld and Charlene Hohl, Department of Medical Biochemistry, Ohio State University; and Dr. E-G. Krause, Max Delbruck Center of Molecular Medicine, Department of Cardiology, Berlin, Germany; Dr. Brian Kobilka, Howard Hughes Medical Institutes, Stanford University Medical Center.


## Steven J. Sollott, M.D.

Investigator, Cardiac Function Section

Gerontology Research Center
Room 3-109
Phone 410-558-8657
Fax 410-558-8150
E mail sollots@grc.nia.nih.gov



**Keywords:**
excitation-contraction coupling
calcium
nitric oxide
chemotaxis


**Recent Publications:**


Sollott SJ, et al. *Am J Physiol* 1996; 271: H896-905.

Miyashita Y, et al. *Am J Physiol* 1996; 271: H244-H255.

Shah AM, et al. *Circ Res* 1997; 80(5): 688-698.

Irani K, et al. *Science* 1997; 275(5306): 1649-1652.


**Biography:** Dr. Sollott received his M.D. from the University of Rochester and completed his residency in internal medicine at a Cornell University program. He subsequently completed his cardiology fellowship at Johns Hopkins University and an NIH medical staff fellowship at LCS, GRC. His research attempts to bridge interests spanning basic and clinical science to therapeutics.

We are studying structure and function of cells from the cardiovascular system along two principal and distinct lines: 1) mechanisms of cardiac contractility, and 2) cellular changes after vascular injury. An underlying theme in both of these areas involves the pursuit and development of single cell biophysical methods to overcome certain limitations and complexities inherent in *in vivo* and in multicellular *in vitro* experimental systems, to gain an understanding of basic cell biological processes that may have implications for the pathophysiology and treatment of human disease.

**Mechanisms of Cardiac Contractility**: Principal research efforts, often employing these newly-developed biophysical methods, have focused on the regulation of contractility in intact cardiac myocytes, with particular emphasis on modulation of myofilament contractile activation by novel signaling pathways, for example, via alterations in the balance of specific kinase/phosphatase pathways, via cross-talk between the cGMP- and cAMP-dependent pathways, and via endogenous nitric oxide-dependent mechanisms. Recent work has focused on novel mechanisms of recruitment of contractile activation underlying the Frank-Starling response.

**Cellular Response to Vascular Injury**: The other major research direction involves the investigation of basic cellular responses of vascular smooth muscle cells during gradient-directed chemotaxis, in order to gain insight into fundamental events in the pathogenesis of vascular disease. These experiments with vascular smooth muscle cells have enabled an understanding of how focal receptor-tyrosine-kinase activation coordinates

the cascade of signaling traffic and the reorganization of the cytoskeleton, leading to directed migration. Migration of vascular smooth muscle cells from the arterial media to the intima is a key event in the pathogenesis of occlusive vascular disorders, including atherosclerosis and post-angioplasty restenosis. We found that a unique intracellular $Ca^{2+}$-signaling profile is initiated via extracellular cues provided specifically by gradient exposure to PDGF, achieving an apparent threshold for activation of CaM kinase II (requisite during VSMC chemotaxis), and this phenomenon mediates VSMC chemotaxis. Differences in this specific $Ca^{2+}$ signaling paradigm among individual cells underlies the asynchronous occurrence rate of chemotaxis seen in VSMC populations. Work is continuing to establish the mechanisms and coordination of subcellular $Ca^{2+}$-microdomains, compartmentalization of CaM kinase II activation and cytoskeletal rearrangements.

These ideas have been applied to the search for strategies to ameliorate the complications of vascular injury. We found that nanomolar levels of paclitaxel (taxol) blocked chemotaxis of VSMC in culture via specific interference with microtubule function, without killing cells. Subsequent *in vivo* experiments showed that paclitaxel, given systemically to rats at doses achieving blood levels some 2 orders of magnitude below that used in oncologic therapeutics (i.e., averaging well below peak levels of 50 nM), reduces the extent of neointimal proliferation following balloon injury by 70-80% without apparent toxicity. Currently, studies in larger mammals are under way to determine the feasibility of initiating a clinical trial in humans. Also, collaborative efforts are under way pursuing local paclitaxel delivery schemes, such as paclitaxel-coated stents, for this purpose. A microtubule-stabilizing-agent use-patent has been obtained for the applications of paclitaxel in treatment of atherosclerosis and restenosis, and a CRADA has been established with private industry partners.

**Collaborators:** Salvatore Pepe, Ph.D., Baker Medical Research Institute, Melbourne, Australia; Kaikobad Irani, M.D., Johns Hopkins University; Pascal Goldschmidt-Clermont, M.D., Ohio State University; Jay L. Zweier, M.D., Johns Hopkins University; Ajay M, Shah, M.D., University of Cardiff, Wales, United Kingdom; Dilip Kittur, M.D., Sc.D., Johns Hopkins University; Robert S. Danziger, M.D., Columbia University; David Wink, Ph.D., NHLBI; Antoine Younes, Ph.D., Universite d’Auvergne Clermont, Aubiere, France.


**Harold A. Spurgeon, Ph.D.**
Senior Staff Scientist, Instrumentation Core Unit

Gerontology Research Center
Room 3-D-08
Phone 410-558-8203
Fax 410-558-8150
E mail spurgeonh@grc.nia.nih.gov



**Keywords:**
cell calcium
cardiac electrophysiology
confocal microscopy
contraction mechanics


**Recent Publications:**


Cheng H, et al. *Cell Calcium* 1996; 20: 129-140.

Sollott SJ, et al. *Am J Physiol* 1996; 40: H896-H905.

Xiao R-P, et al. *Am J Physiol* 1997; 41: H797-H805.

Wheeler DM, et al. *Anesthesiology* 1997; 86(1): 137-146.


**Biography:** Dr. Harold A. Spurgeon received his Ph.D. in Medical Physiology from Loyola University Stritch School of Medicine, Chicago, in 1972, and completed postdoctoral training at the University of New Mexico, Albuquerque. He joined the Intramural Research Program in 1974 where he worked on problems related to cardiac muscle mechanics and innervation control.

**Recent Research:** Because the unique characteristics of our implementation of a system to measure calcium, cell shortening, and membrane current/voltage, we measure the true instantaneous values associated with each of these parameters, without “time smearing” during a given excitation/contraction cycle due to averaging effects. This approach has allowed us to investigate the codependency of cell length, free calcium, or potential, in a two dimensional model. Clearly there is a time-dependence as well, important in understanding the interaction between time-dependent changes in the extent of cell shortening and free intracellular calcium.

Confocal imaging opens up exciting windows into physiological questions. For example, calcium “sparks” recently described in *Science*, raise questions about the role of transmembrane currents accompanying microburst releases of calcium in highly discrete regions of the cell. We hope to determine conclusively whether the sparks result in an evoked localized calcium current triggered by the local release of calcium from (most likely) the sarcoplasmic reticulum, or whether the sparks are themselves triggered by localized transient increases in calcium conductance leading to localized SR release.

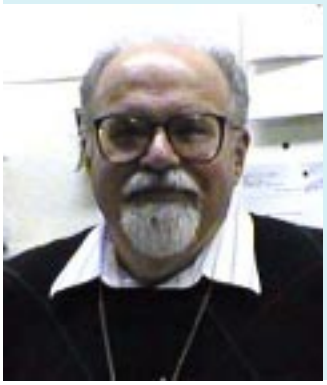
Image processing is in its infancy. Individual images contain large amounts of information which must basically be hand-tweaked to extract even a small fraction of the information contained. As imaging of a variety of types becomes even more prevalent, expert systems of software and hardware offer the only currently identifiable relief in processing this mountain of data. We will need to develop and integrate these approaches

to increase our data reduction efficiency. Quantification of image characteristics has been likewise largely confined to “looks like, bigger than,” although very recently more quantitative descriptors have begun to appear. To understand the physiology, functional descriptors need to be developed as well. Pattern recognition techniques and fractal reduction may prove useful here.

In addition to the core technical development tasks and assorted research projects, I have become involved in human studies. By adding pulsewave measurements to two existing multi-center studies being conducted by NHLBI and our Human Studies Unit, we gain a relatively low cost access to approximately 3800 subjects already screened as hypertensive. We will be able to follow these patients in a blind interventional study where the intervention planned is prescribed exercise. We hope to produce a more comprehensive picture of arterial stiffness, aging, and disease, particularly in the important area of structure/function relationships as they relate to disease outcomes. By building a unified database across several of these studies, the bias inherent in single population studies should be eliminated. In the BLSA population in particular, both prospective and retrospective data are available for disease outcomes, but the somewhat biased population demographics preclude more global interpretations. We hope as well to get better data relative to dietary sodium intake in at least a subset of these populations, for the role of increased sodium in modifying vascular stiffness is not clear.

Further investigations in the pharmacology of $ß_1$ and $ß_2$AR are being extended to address, in the dog model, the changes in phosphorylation of key cellular proteins by $ß_1$ and $ß_2$AR. Of particular importance is definition of the $ß_2$ inhibitory pathway in this model. The preponderance of evidence points to a non-cAMP mediated mechanism for $ß_2$ activation because there is no evidence to date showing increased cAMP production in the adult dog heart by I.C. zinterol, $ß_2$ agonist, and no phospholamban phosphorylation. In collaboration with Dr. George Krause, who has developed a monoclonal antibody for the ß-subunit of the L-type calcium channel we will further define ß-adrenergic subtype actions on the cardiac L-type calcium channel.

**Collaborators:** Gary Gerstenblith, M.D., Johns Hopkins School of Medicine; Peter Snell, Ph.D., University of Texas Southwestern; Kim Sutton-Tyrrell, Ph.D., University of Pittsburgh; Leslie Pruitt, Ph.D., Stanford University; Paul Ribisl, Ph.D., Wake Forest University; Mary O'Toole, Ph.D., University of Tennessee; Peter Vaitkevicius, M.D., Johns Hopkins School of Medicine; Richard Havlik, M.D., Epidemiology Demography and Biometry Program, IRP, NIA; Mark Lane, Ph.D., Laboratory of Cellular and Molecular Biology, IRP, NIA; Salvatore Pepe, Ph.D., Baker Research Institute, Australia; A.J. Shah, M.D., University of Wales; Constantine Bogdanov, Ph.D., Cardiology Research Center, Moscow; G. Krause, Ph.D., Max Delbruck Center for Molecular Cardiology, Berlin.




## Mark Talan, M.D., Ph.D.

Medical Officer, Gene Therapy Unit

Gerontology Research Center
Room 3-B-12
Phone 410-558-8214
Fax 410-558-8233
E mail markt@vax.grc.nia.nih.gov



**Keywords:**
thermoregulation
hemodynamics
microcirculation
angiogenesis


**Recent Publications:**


Talan MI, et al. *Physiol Behav* 1996; 60: 1285-1289.

Talan MI, et al. *Exp Gerontol* 1996; 31: 687-698.

Talan MI, *Ann NY Acad Sci* 1997; 813: 95-100.

Shefer VI, et al. *Exp Gerontol* 1997; 32: 325-332.


**Biography:** Dr. Talan was trained as a physician at the First Leningrad Medical School in Russia. He received his Ph.D. in Physiology at the Pavlov Institute of Physiology in Russia where he continued to work as a scientist before coming to the NIA in 1980. His studies at the NIA in the area of thermoregulation, regulation of hemodynamics, and operant conditioning of autonomic function evolved to his present interests concerning (1) the effects of thermoregulatory responses to cold on the development of hypertension and other cardiovascular risk factors; and (2) microcirculation and stimulation of angiogenesis.

**The Mechanisms of Cold-Induced Hypertension**: A number of epidemiological observations reported an increased incidence of adverse cardiovascular events and high prevalence of elevated arterial blood pressure during the winter. The entire population is affected by this annual rhythm but the elderly are the most vulnerable to the negative effects of seasonal changes. The colder ambient temperature was implicated as the single most important factor responsible for this effect, but the mechanisms of seasonal hypertension and elevation of other cardiovascular risk factors remain poorly understood. This program was set up to develop an experimental animal model of cold-induced hypertension and to investigate the mechanisms responsible for elevation of blood pressure and other risk factors during cold acclimation.

The immediate goal of this project is to define the time course and parameters of changes in arterial blood pressure associated with acclimation to different environmental temperatures and to measure accompanied changes in plasma volume and rheological characteristics of blood in adult and aged Wistar rats.

Adult and aged Wistar rats acclimated to thermoneutrality, i.e. temperature that does not requires any metabolic expenditure to maintain body temperature (26EC), were exposed to cold (6EC) for 9 weeks followed by 5 weeks of rewarming (26EC). In adult rats the elevation of systolic blood pressure started two weeks after beginning of cold exposure and reached 30 mmHg above the control level after six weeks of exposure. The elevation of blood pressure was preceded by a 50% plasma volume expansion and was accompanied by an increased water consumption and elevation of whole blood viscosity. During the five weeks of rewarming plasma volume and water consumption returned to normal but blood pressure and blood viscosity remained elevated. Cold exposure of aged rats did not result in elevation of the systolic blood pressure, blood viscosity or plasma volume expansion, however, before cold exposure these parameters were already higher in aged than in adult rats.

We believe that cold-induced elevation of blood pressure in rats represents the first naturalistic experimental model of volume-associated hypertension that will facilitate the development of treatment and prevention of this debilitating condition. We will be carrying out systematic studies to further understand the mechanisms by which naturally occurring physiological responses to cold might contribute to changes in circulating plasma volume, blood viscosity, and eventually lead to morphological vascular changes characteristic for hypertension.

**Collaborators:** Natalya Roukoyatkina, Ph.D., Behavioral Hypertension Section, LCS; Joseph Rifkind, Ph.D., and Ranjeet Ajmani, Ph.D., Molecular Dynamic Section, LCMB.


**Jeffrey Froehlich, M.D.**
Medical Officer, Chief, Membrane Biology Section

Gerontology Research Center
Room 3-E-12
Phone 410-558-8205
Fax 410-558-8150
E mail jeffro@vax.grc.nia.nih.gov



**Keywords:**
ion transport
relaxation
restenosis
local delivery


**Recent Publications:**


Miyashita Y, et al. *Am J Physiol* 1997; 272 : H244-H255.

Kane DJ, et al. *Biochemistry* 1997; 36(43): 13406-13420.

Hartung K, et al. *Biophys J* 1997; 72(6): 2503-2514.


**Biography:** Dr. Froehlich received his M.D. degree from the University of Chicago in 1969 and completed 3 years of postgraduate research in the Department of Biophysics before joining the NIH as a Commissioned Officer in the USPHS. In 1985 he was named chief of the Membrane Biology Section which became part of the Laboratory of Cardiovascular Science, NIA, in 1990. At the NIH, he collaborated with Robert Berger to develop a rapid mixing, chemical quenched-flow device that has been extensively used for kinetic characterization of the ion motive ATPases, an interest that evolved from his postgraduate work. Although internationally recognized for his contributions to the field of active transport in subcellular organelles, he has also studied $Ca^{2+}$ transport in vascular smooth muscle cells, focusing on $ß_2$-agonist-mediated relaxation and aging. Recently, he has begun to examine the vascular smooth muscle response to mechanical injury in a CRADA-supported program aimed at developing pharmacological approaches for the prevention of restenosis following angioplasty.

**Ion Motive ATPases**: The ion motive ATPases represent an important class of enzymes that couple ATP hydrolysis to unidirectional, uphill cation transport. A major theme in Dr. Froehlich's research on these enzymes in eukaryotic cells has been the role of quaternary (subunit-subunit) protein interactions in the mechanism of ion-dependent ATP hydrolysis. Using rapid mixing techniques (quenched-flow and stopped-flow mixing), he has identified specific features of the pre-steady state and steady state kinetic behavior of these enzymes that reflect the presence of subunit-subunit interactions in the catalytic mechanism. These interactions impose constraints on the timing of the reactions in the conformationally coupled subunits, forcing them to occur sequentially rather than simultaneously. In a dimeric enzyme, the leading protomer binds the transported ion and moves it across the membrane in advance of these events in the neighboring protomer. A central problem has been to understand why the system operates this way as opposed to rapidly transporting all of the

bound cations across the membrane, yielding the highest catalytic efficiency. An answer to this problem may be found in the conservation of free energy which can be transferred between adjacent subunits residing at different energy levels. Another explanation involves the minimization of repulsive forces between like charges which arise from complexation of the transported cation with the pump protein. These and other issues related to the oligomeric behavior of these enzymes are being explored by a variety of special techniques including rapid chemical quenching, the laser flash/lipid bilayer technique and time-resolved electron spin resonance. Future studies involving prokaryotic ion motive ATPases, which exhibit similar kinetic behavior, will allow testing of these hypotheses by site-directed mutagenesis.

**Vascular Smooth Muscle Relaxation Mechanisms:** Older individuals manifest an increase in systolic blood pressure together with increased arterial stiffening and reduced vasorelaxation in response to $ß_2$-adrenergic agonists. Dr. Froehlich has proposed a common etiology for these changes based on altered smooth muscle $Ca^{2+}$ metabolism and has tested this hypothesis using freshly-isolated rat arterial cells loaded with a cytoplasmic $Ca^{2+}$ indicator and ratiometric video fluorescence imaging. The $ß_2$-agonist, isoproterenol (ISO), was found to mediate smooth muscle relaxation by reducing $Ca^{2+}$ influx and by decreasing $Ca^{2+}$ stores in the sarcoplasmic reticulum (SR). These effects resulted from cyclic AMP-dependent stimulation of the $Na^+/K^+$ pump and secondary activation of the $Na^+/Ca^{2+}$ exchanger. Older cells exhibited larger SR $Ca^{2+}$ stores following ISO, which reduced the $Ca^{2+}$ buffering capacity of SR and increased the probability of enhanced vascular tone. Increased arterial tonus might explain the arterial stiffening and rise in systolic blood pressure associated with aging. The single cell model affords a unique opportunity to explore the relationship between intracellular $Ca^{2+}$ and contractility in smooth muscle pharmacomechanical coupling.

**Local Drug Delivery and Restenosis:** A complication facing 40-50% of the patients undergoing percutaneous transluminal coronary angioplasty (PTCA) for symptomatic coronary artery disease is restenosis, a condition associated with neointimal proliferation and late vascular remodeling. Efforts to develop a pharmacological approach for the prevention of restenosis have focused on paclitaxel, an anti-neoplastic drug with proven efficacy at preventing vascular smooth muscle cell migration and proliferation. Paclitaxel coated directly onto metallic intracoronary stents was shown to produce a significant reduction in neointimal hyperplasia and lumenal encroachment in minipigs without the thrombotic complications commonly associated with some polymeric local delivery systems. Future research will concentrate on defining a safe and effective therapeutic dosing range in preparation for clinical trials.

**Collaborators:** R. W. Albers, NINCDS, NIH; A. Heldman, J. Brinker, D. Kittur, R. Hruban, G. Jenkins, Johns Hopkins University; K. Fendler, K. Hartung, E. Bamberg, R. Clarke, Max-Planck-Institute for Biophysics; K. Altendorf, S. Droese, Osnabrueck University; W. Epstein, University of Chicago; K. Taniguchi, Hokkaido University.


## Linda Cheng, Ph.D.
Research Chemist, Membrane Biology Section

Gerontology Research Center
Room 3-E-15
Phone 410-558-8683
Fax 410-558-8150
E mail chengl@grc.nia.nih.gov



**Keywords:**
vascular injury
restenosis
drug delivery
gene therapy


**Recent Publications:**


Yamamoto H, et al. *Exp Cell Res* 1996; 222: 125-130.

Li Z, et al. *Am J Path* 1996; 148: 121-128.

Miyashita Y, et al. *Am J Physiol* 1997; 272: H244-H255.


**Biography:** Dr. Cheng received her Ph.D. in biochemistry from Wayne State University. After completing her postdoctoral fellowship at The Johns Hopkins University, she joined the NIA in 1977. Her present interests focus on using gene therapy and paclitaxel to reduce neointimal development after vascular injury in animal models of restenosis.

**Animal Model of Restenosis:** Mechanical injury of the blood vessel wall causes de-endotheliazation, mural thrombosis, platelet activation, thrombin generation, and the release of growth factors and cytokines. These events lead to vascular smooth muscle cell migration, proliferation and extracellular matrix deposition that are the hallmarks of restenosis. Our laboratory has been involved in developing the rat and pig models of restenosis and using these models to understand *in vivo* proteolytic cascades and intracellular signal transduction. In addition, we use these models to develop therapeutic strategies such as local drug delivery and gene therapy to treat restenosis.

**Local Drug Delivery:** Local delivery of therapeutic agents to the arterial wall represents a new strategy for the treatment of fibroproliferative vascular disease, including restenosis after percutaneous transluminal angioplasty and arterio-venous bypass grafting. A major advantage of local delivery is the ability to achieve effective tissue concentrations of the drug while avoiding the toxic effects associated with systemic dosing. We developed biodegradable microbeads as a mechanism of local delivery. Our microbeads are formed by the complex coacervation of collagen and chrondroitin sulfate. Microbeads formed by this process are particularly suitable for entrapping proteins and hydrophobic com-

pounds. We have shown that paclitaxel, an antineoplastic agent, inhibited neointimal hyperplasia resulting from balloon catheter injury when administered systemically to rats. The ability of locally-administered paclitaxel to prevent the neointimal hyperplastic response was tested by incorporating the drug into sustained-release biodegradable microbeads which were applied to the adventitial surface of the rat carotid artery immediately following balloon injury. Locally-delivered paclitaxel produced a dose-dependent and site-specific inhibition of neointimal growth. This inhibitory effect could be achieved without a detectable inflammatory response and without evidence of medial wall cell death at low paclitaxel concentrations. These results demonstrate complete inhibition of neointimal hyperplasia by extravascular paclitaxel applied directly over the site of injury. Local delivery of paclitaxel in biodegradable polymer matrices may have application in the creation of arterio-venous shunts and in other surgical grafting procedures where stenosis is a complication. Our current efforts concentrate on the effect of paclitaxel coated stents on pig coronary arteries.

**Gene Therapy:** The migration of vascular smooth muscle cells (VSMCs) is an important and essential step in restenosis after balloon angioplasty. This step is dependent on the local degradation and remodeling of the extracellular matrix. We have shown that vascular injury increased the expression and activation of matrix metalloproteinase 2 (MMP-2) during the migration of VSMC to the intima in the rat carotid artery injury. Adenoviral vectors are a very useful tool for the transfer of genetic material to the vessel wall. In order to inhibit VSMC migration from media to the intima, we constructed a replication-deficient adenovirus vector carrying the cDNA for human tissue inhibitor of metalloproteinase-2 (AdCMV.hTIMP-2). TIMP-2 is a physiologic MMP-2 inhibitor. AdCMV.hTIMP-2 was previously shown to inhibit MMP-2 activity and SMC invasion in cultured VSMC. Recently, we have shown that medial VSMCs exhibit positive staining for human TIMP-2 protein after vascular injury and infection with AdCMV.hTIMP-2. In addition, we demonstrated that localized arterial infection with AdCMV.hTIMP-2 at the time of vascular injury significantly reduced the number of cells in the intima and neointimal formation. These results demonstrate the important role of TIMP-2 in regulating migration of VSMC, and suggest either TIMP-2 alone or in combination may have therapeutic potential for treating restenosis after vascular injury.

**Collaborators:** Kam Leong, Ph.D., Johns Hopkins University.


**David E. Anderson, Ph.D.**
Chief, Behavioral Hypertension Section

Gerontology Research Center
Room 3-B-13
Phone 410-558-8213
Fax 410-558-8233
E mail danderso@grc.nia.nih.gov



**Keywords:**
carbon dioxide
endogneous digitalis-like factors
hypertension
sodium


**Recent Publications:**


Anderson DE, et al. *Journal of Hypertension* 1996; 14: 1073-1079.

Fedorova OV, et al. *American Journal of Hypertension* 1996; 9: 1126-1131.

Dhokalia A, et al. *Psychosomatic Medicine* 1997, In Press.

Fedorova OV, et al. *American Journal of Hypertension* 1997; 10: 929-935.


**Biography:** Dr. David E. Anderson received his Ph.D. from the University of Oregon in 1966. He developed his career interest in the environmental and behavioral origins of hypertension and on the nature of the mediating physiological mechanisms at The Johns Hopkins University School of Medicine (1968-1981) and the University of South Florida (1981-1987). During that period, he was a recipient of an NIH Research Career Development Award (1983-1987) and the Pavlovian Award for Biological Science in 1985. He came to the National Institute on Aging in 1987, and was appointed Chief of the Behavioral Hypertension Section of the Laboratory of Cardiovascular Sciences in 1997.

**The Behavioral Origins of Hypertension:** The goal of the Behavioral Hypertension Section is to clarify how interactions of the individual with the external environment contribute to the development of chronic hypertension. It is known that intermittent behavioral stress can potentiate a sodium-sensitive experimental hypertension in large laboratory animals. This form of hypertension involves sustained renal sodium retention, but its development is not prevented by renal denervation. The finding that sustained suppression of breathing is characteristic of hypertensive animals led to a focus in this Section on behaviorally-induced increases in $pCO_2$ and its effects on renal regulation of sodium, sodium pump inhibitors and blood pressure. A fundamental hypothesis that directs experimental work is that behaviorally induced breathing suppression increases plasma volume via increases in $pCO_2$, carbonic acid formation, hydrogen ion concentrations, and increased renal sodium-hydrogen exchange, and potentiates the hypertensive effects of a high sodium diet.

**Behaviorally Induced Respiratory Suppression and Blood Pressure Regulation**: In support of this view, one series of studies with laboratory micropigs showed that anticipatory behavioral stress can increase $pCO_2$ acutely, decrease plasma pH, increase plasma bicarbonate, and decrease hematocrit, indicative of plasma volume expansion. Consistent with this finding is the associated observation that endogenous digitalis-like factors

(EDLF) which are sensitive to plasma volume changes are also increased under these conditions. A parallel series of studies with healthy humans showed that comparable effects could be produced by self-regulated inhibition by breathing maintained by a respiratory gas monitor and feedback system.

More recent studies have shown that high resting end tidal $CO_2$ is a risk factor for sodium sensitivity in older, and to a lesser extent, in younger humans. These studies also showed that urinary EDLF excretion was higher in subjects with high end tidal $CO_2$. Resting end tidal $CO_2$ is correlated with $pCO_2$ in humans with healthy lungs and can be measured nonintrusively and continously. Studies have shown that high resting $PetCO_2$ is stable over time, and highly positively correlated with the tendency to worry and experience negative affects. Thus, high resting $pCO_2$ may be an indicator of chronic stress in humans.

**Endogenous Digitalis-like Factors in Blood Pressure Regulation:** Studies in this Section also focus on the role of EDLF in blood pressure regulation. EDLF are of interest in this laboratory because they vary with plasma volume and can inhibit sodium/potassium pump activity in vascular smooth muscle. Previous work in this laboratory showed that an endogenous marinobufagenin-like bufodienolide is a more rapid and powerful vasoconstrictor than an endogenous ouabain-like cardenolide, and that plasma concentrations of each are stimulated by saline-induced expansion of plasma volume. Moreover, a high sodium diet can sustain increases in urinary marinobufagenin excretion for at least weeks. Studies have also shown that the bufodienolide has a greater affinity for the $\alpha$-1 isoform of Na,K-ATPase, which is concentrated in vascular smooth muscle membranes, and that the cardenolide has a greater affinity for the $\alpha$-3 isoform, which is concentrated in neural membranes. Thus, the two EDLF may have different primary sites of action and different roles in blood pressure regulation. Studies will be conducted to determine the effects of administration of EDLF antibodies and natural protective ligands of Na, K-ATPase in various models of hypertension.

**Ongoing Studies**: Current work focuses on the development of a model of behavioral hypertension in the rat which will test the hypothesis that sodium sensitivity is a function of habitual breathing pattern and chronic increases in $pCO_2$. Rats maintained in a metabolic chamber are trained to self regulate breathing rates for up to eight hours per day, to avoid onset of

aversive bright light. Blood pressure is monitored continuously 24 hr per day via telemetry. It is hypothesized that chronic breathing inhibition will potentiate the development of experimental hypertension in rats on a high sodium diet, but that this form of hypertension will not occur in sodium-fed rats who maintain high breathing rates or in breathing-suppressed rats on a low sodium diet. The role of EDLF, sodium pump activity and sodium-hydrogen exchange in the development of this form of hypertension will be analyzed experimentally.

Finally, studies are in progress with participants in the Baltimore Longitudinal Study on Aging to examine the relationships between breathing patterns, resting $pCO_2$, plasma volume, renal functions, and long-term regulation of blood pressure. Taken together, these studies may clarify how habitual patterns of behavioral interactions contribute to sodium sensitivity and the development of hypertension, and provide the basis for prevention or delay of this common cardiovascular disorder and its sequelae.

**Collaborators:** Alexei Y. Bagrov, M.D., Ph.D., Sechenov Institute of Evolutionary Physiology, Russian Academy of Sciences, St. Petersburg, Russia; A. Dhokalia, Ph.D., Laboratory of Cardiovascular Sciences, NIA; Olga V. Fedorova, Ph.D., Laboratory of Cardiovascular Sciences, NIA; Richard G. Weissman, Ph.D., Laboratory of Cardiovascular Sciences, NIA.